Cardiovascular tissue engineering: state of the art

In patients requiring coronary or peripheral vascular bypass procedures, autogenous arterial or vein grafts remain as the conduit of choice even in the case of redo patients. It is in this class of redo patients that often natural tissue of suitable quality becomes unavailable; so that prosthetic material is then used. Prosthetic grafts are liable to fail due to graft occlusion caused by surface thrombogenicity and lack of elasticity. To prevent this, seeding of the graft lumen with endothelial cells has been undertaken and recent clinical studies have evidenced patency rates approaching reasonable vein grafts. Recent advances have also looked at developing a completely artificial biological graft engineered from the patient’s cells with surface and viscoelastic properties similar to autogenous vessels. This review encompasses both endothelialisation of grafts and the construction of biological cardiovascular conduits.

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Résumé

Les veines ou artères autologues restent le matériau de remplacement de choix pour les pontages coronariens ou périphériques, même chez les patients dont l’état de santé nécessite une ré-intervention. Cependant, pour ces derniers, l’utilisation d’un tel matériau s’avère souvent impossible et l’implantation d’une prothèse synthétique reste la seule alternative. Les implants synthétiques sont toutefois sujet à l’occlusion en raison de leur caractère thrombogène et de leur manque de compliance. C’est pourquoi des études de recouvrement de leur surface interne par un endothélium vasculaire ont été entreprises, qui aboutissent à des taux de perméabilité avoisinant ceux rapportés pour la veine saphène autologue. Des tentatives de production de vaisseaux biologiques à partir des cellules des patients sont également en cours, lesquelles présenteraient des propriétés visco-élastiques améliorées par rapport à leurs contre-parties synthétiques. Cet article fait le point sur l’endothélialisation des implants synthétiques et des diverses stratégies de fabrication des implants biologiques.

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Keywords: Cardiovascular; Coronary bypass grafts; Endothelium; Hybrid artificial organ; Tissue-engineering; Vascular bypass grafts

Mots clés : Cardiovasculaire ; Endothélium ; Génie tissulaire ; Organe artificiel hybride ; Pontages coronariens ; Pontages vasculaires périfériques

1. Introduction

Coronary and peripheral vascular bypass grafting is now performed in more than 1 million cases annually in the United States and Europe. Nevertheless, it is not without significant constraints or complications [1–5], for instance, vein graft disease [6]. Autogenous saphenous or an arm vein is the current material of choice for use as a bypass graft in infragastrual arterial reconstruction for peripheral bypass procedure while autologous vessels such as the internal mammary...
artery and the long saphenous vein are used in cardiac bypass procedures. Some patients undergo bypass with prosthetic grafts because no suitable vessel is available, due to previous operations where it has been already used, this class of patient being termed redo, or the remaining vessels are of poor quality. Unfortunately, replacement of arteries with purely synthetic polymeric conduits often leads to the failure of such grafts. This is accentuated in small diameter (less than 6 mm) grafts or in areas of low-flow. This is especially evident in below knee vascular prostheses or in coronary artery bypass grafts (CABG), where very high-flow rates are essential. This is due to the thrombogenicity of the internal surface of the graft and the formation and growth of intimal hyperplasia (IH) around the anastomoses. The latter is due to compliance mismatch between the relatively non-elastic graft and the native viscoelastic blood vessel, and the damage to the endothelium by the sutures of the anastomoses.

The principal polymeric graft materials used in peripheral vascular reconstructions are woven polyethylene terephthalate (Dacron) and expanded polytetrafluoroethylene (ePTFE) while cardiac surgeons use ePTFE graft though reluctantly in some centres. The poor mechanical characteristics of such polymeric materials in term of its size and compliance are significant factors which contribute to their poor patency.

The other important factor implicated in graft failure is the lack of endothelial cells (ECs) lining the lumen of the graft. This endothelial monolayer that lines the normal blood vessel serves as a bioregulator of cardiovascular physiology, a part of Virchow’s triad. The endothelium provides structural integrity to the blood vessel by forming a continuous selectively permeable, thromboresistant barrier between circulating blood and the arterial wall. It also controls blood flow and vessel tone, platelet activation, adhesion between circulating blood and the arterial wall. It also provides structural integrity to the blood vessel by forming a continuous selectively permeable, thromboresistant barrier between circulating blood and the arterial wall. It also controls blood flow and vessel tone, platelet activation, adhesion and aggregation, leukocyte adhesion and smooth muscle cell (SMC) migration and proliferation.

The in vitro process of lining ECs to the lumen of the graft is known as ‘seeding’ [16–18]. To be successful, seeding of grafts has required culturing of ECs over a period of weeks to date. As a result of this problem, there have been numerous attempts at creating fully tissue-engineered vessels composed of prosthetic (ePTFE, Dacron or polyurethane), biore呾orable (e.g. PGA, PLLA) or fully biological materials together with autologous cells, which can be readily available on the shelf of any operating theatre.

The principal goal of this review is to highlight the current clinical perspectives in the development of a biomimetic vascular substitute that possesses both the mechanical and functional qualities required by both cardiothoracic and vascular surgeons for bypass surgery. Specifically, we seek to emphasise the recent advances that have taken place in cell seeding as well as tissue engineering of the current generation of prosthetic grafts.

2. Search methods

All the papers were identified by PubMed and CAS searches between years 1966 and 2004 with the following keywords: Endothelium, Tissue-Engineering, Coronary bypass grafts, Vascular bypass grafts, Seeding, Mesothelium, Cardiovascular, Prosthetic graft, Biological tissue-engineered vascular grafts, Tissue-engineered vascular grafts, Biological vascular grafts, Endothelial progenitor cells (EPCs).

3. The need for tissue engineering of prosthetic grafts

The ideal cardiovascular bypass graft must have the following qualities: durability, resistance to degradation, non-toxicity, resistance to infection and availability in a variety of sizes which suits a wide range of cardiac and peripheral vascular reconstructions. In addition, the implant should have good handling characteristics, be flexible, easy to suture and result in minimal needle-hole and interstitial bleeding following implantation. For long-term use, the prosthesis must generate an optimum tissue reaction to allow healing whilst preventing fibrous capsule formation. Lastly, the ideal bypass graft must have similar viscoelastic properties to that of the host artery and be completely non-thrombogenic even in low-flow states, the two most important causes of graft failure.

Unfortunately the currently available prosthetic vascular grafts, Dacron and ePTFE and in the case of coronary applications ePTFE alone are thrombogenic and have poor compliance. Polyurethane grafts which have been available for the last 40 years have characteristics that would be ideal for use in bypass procedures namely similar compliance to native arteries with a surface that is conducive for seeding.

Unfortunately, polyurethane grafts have had variable results clinically with a tendency to degrade causing aneurysm formation. The site of the degradation of the polyurethane graft is in the amorphous soft segment typically ester or ether. These segments degrade rapidly either due to hydrolysis or oxidative degradation. Hard polyurethanes which are low in ether content can function for long periods with virtually no degradation while the reverse is true for high content urethanes, however these have limited usage of bypass conduits as they are relatively stiff.

We have developed a polyurethane (CPU) based on polycarbonate-urea soft segments with similar elastic properties to native arteries. This material has proven to be very resistant to degradation in short to mid-term in vitro and in vivo studies. Results of an ongoing multicentre clinical trial(s) are being eagerly awaited.

Other approaches can involve the fabrication of scaffolds based on natural biomaterials such as collagen where the biomaterial has intrinsic and specific biological activities which influence cellular interactions. The major disadvantage of using natural biomaterials is that they are generally not
easily modified and have limited physical and chemical properties. Alternatively a bio-hybrid lumen may be synthesised. As shown in Fig. 1, cells invade and assimilate into a scaffold made of collagen gel and form a bioactive scaffold. The obvious worry is whether such grafts would hold up to the high pressure environment of arterial flow. To this end, these grafts have been cross-linked with glutaraldehyde and the initial results are promising [30].

Specific factors or molecules preventing thrombosis may also be incorporated into the luminal surface of the prosthetic graft surface. However, it must be said that a completely biological graft with either autologous tissue alone or a mixture of autologous and heterologous tissues would more closely resemble healthy functional vessels.

This review focuses on the latter approaches namely, bio-hybrid and completely biological vessels [31]. Table 1 shows the current and the possible future plethora of bypass grafts which are or will be in used in cardiac and peripheral vascular bypass procedures.

### 3.1. Development of hybrid/seeded graft

Prosthetic grafts do not spontaneously endothelialise in humans except for a short peri-anastomotic region, where the anastomosis is made [32,33]. It is thought that this lack of ECs contributes to the failure of the prosthetic graft by thrombosis in this region devoid of cells [10]. A cellular engineering approach called ‘seeding’ has been used to overcome this problem. In simple terms, it involves lining the lumen of the graft with ECs [34].

#### 3.1.1. Seeding techniques with mature ECs

There are currently two strategies in seeding of grafts: a two-stage or a single-stage procedure [16,18,24]. Two-stage seeding is the conventional and successful technique used and is discussed below. Single-stage seeding is the technique most aspired for and this is discussed subsequently. The principal sources for EC extraction are vein, artery, omental, subcutaneous fat, laparotomy washes and blood as progenitor cells, tissues which are easily accessible in clinical practice [35].

##### 3.1.1.1. Two-stage seeding

In two-stage seeding, the ECs are extracted and made to undergo a prolonged period of cell culture in the laboratory in order to increase the cell numbers before seeding. The cells are normally cultured for a period of 2–4 weeks [36]. The main source of ECs in two-stage seeding is a vein from the patient though arteries are used occasionally. This technique has given rise to excellent patency rates in animals: patency rates of 62.5% without antiplatelet drugs and 100% with them [37–40]. The high initial density of seeded ECs means that even with cell loss on exposure to pulsatile blood flow on implantation, the ECs are still functionally effective as a whole since a relatively high number of cells are still left attached. However a confounding factor is that in animals, such constructs tend to show improved results. This is due to the inherent ability of the animals to self-endothelialise their vascular lumens [41], a problem which was only realised when initial clinical trials in humans with these seeded grafts showed poor results. This problem may be overcome if older animals are used. In a recent experiment, Noishiki and co-workers showed that the graft healing was delayed in older animals as compared to their younger ones. This model would mimic the subset of older patients who frequently need vascular bypass surgeries [42,43].

![Fig. 1. The use of a collagen gel construct as a temporary scaffold for the induction of cells [30].](image)

<table>
<thead>
<tr>
<th>Graft Type</th>
<th>Applied experimentally</th>
<th>Under clinical trial</th>
<th>Used routinely</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autogenous vein b</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Radial artery [184]</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Internal mammary artery [185]</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Gastro-epiploic artery [186]</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>EPTFE</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Dacron</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CPU</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Tissue engineering</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seeded/hybrid</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Living graft</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

EPTFE: expanded polytetrafluoroethylene; Dacron: polyethylene terephthalate; CPU: compliant polyurethane graft.

* Clinical trial imminent.

b Saphenous or arm vein.

c Not yet.
thermore, this also holds true for neoointima formation (a sign of tissue healing) and is further compounded by the type of graft used [44–46]. It is for this reason that the authors tend to assess the efficacy of their seeding experiments in vitro with the use of physiological blood flow pumps [24,47].

3.1.1.2. Clinical trials. An apparent disadvantage of the two-stage seeding procedure in clinical practice is that it cannot be used in the emergency situation because of the prolonged cell culture time (2–4 weeks), the ever-increasing probability of infection within the cell culture medium and the inability of the cells to proliferate effectively with time [48,49]. In addition, it incurs the extra cost of a cell culture technician and the need for a culture laboratory, and in non-teaching hospitals this is simply not possible.

Clinical trials involving the two-stage in vitro endothelialisation of vascular grafts prior to implantation have shown conclusively that tissue engineering results in a significantly enhanced clinical performance in small diameter grafts. This is an example of the clinical application of tissue engineering, bridging the gap between the prosthetic graft and the autologous vein graft. Another successful trial is the two-stage seeding of 3 mm ePTFE coronary grafts by Laube et al. [50]. Their results show a 90.5% vessel patency rate 52 weeks post-implantation [50]. No two-stage seeding trials using fat have been undertaken so far, or using progenitor cells.

3.1.1.3. Single-stage seeding. This method also utilises freshly harvested ECs of either venous or microvascular origin [35]. Microvascular sources include omental and subcutaneous fat [51,52]. In the single-stage procedure, the ECs are harvested and then immediately lined onto grafts. The obvious benefit of this is that it can be performed during a surgical operation, thus avoiding a second procedure.

Herring et al. [16] introduced the concept of single-stage seeding in 1978 using ECs derived from canine veins. They showed that grafts seeded by this way in a canine animal model established an extensive EC lining [16]. These successful experiments stimulated a host of other single-stage seeding trials in animal models [53–56]. Single-stage seeding in animals using fat also showed improved patency rates [57]. However for reasons mentioned above, animal models may not be the most suitable method of assessing the efficacy of seeding.

3.1.1.4. Clinical trials. Herring et al. [58] showed that EC seeding in humans will also result in endothelialisation. The results of the initial clinical trials of single-stage seeding using vein as a source of ECs were disappointing [17,18,45,48,59–62]. The reason was thought to be the low seeding density of ECs due to the low numbers of ECs which can be extracted from veins using current methods. Therefore when undertaken, these low numbers exert a minimal effect on the endothelialisation of the PTFE graft [48,49]. This is because a proportion of seeded cells are lost when exposed to pulsatile blood flow [63,64]. The answer to this dilemma is to improve the cell density. This may be accomplished by a process called ‘sodding’. Based on the assumption that ‘sodding’ is needed to improve graft patency, other sources of ECs were evaluated in humans. The principal choice for this is fat as large amounts of cells (>1 x 10^6 per g) can be easily extracted [65]. If subcutaneous fat is used, it can be easily removed by a small abdominal incision or by liposuction. Liposuction has the advantage of a smaller incision and a better yield of ECs due to the increased surface area that is available for enzymatic digestion. EC from fat has been extracted from the abdomen, thigh, buttock and even breast tissue.

The commonly used techniques for EC extraction in these cases are centrifugation and percoll extraction [66]. When percoll is used for cell purification, there is a poor expression of EC markers by these cells and it is therefore a detrimental technique [66]. Both percoll and filtration significantly reduce the number of ECs that can be extracted [67,68]. Overpurifying microvascular cells (MVEC) into a homologous medium may have no beneficial effect as current thinking suggests that a heterogenous MVEC suspension may be equally effective [69].

Unfortunately, clinical trials using subcutaneous fat have so far shown inferior results as compared to two-stage seeding using veins [70]. An alternative source to subcutaneous fat is omental fat [71]. However, this would subject patients to an unnecessary laparotomy and its consequent morbidity.

Cells extracted from omentum have been characterised as mesothelial cells (MCs) by some authors whilst others still regard these as ECs [51]. In any case, both ECs and MCs have similar functional properties, for instance both release anticoagulant substances. Nevertheless, differences are present. For instance, in ePTFE vascular grafts coated with fibroblastic matrix, it was found that more MCs were significantly retained on the matrix-based surface in comparison to ECs [72].

Most clinical trials on single-stage seeding have been performed on small numbers of patients with variable results [17,48,59–62,73–75]. In addition, even fewer clinical trials of single-stage seeding using adipose tissue have been conducted compared to its two-stage counterpart [76].

The major trials of both types of seeding are summarised in Table 2. In a same way stents have also been seeded with ECs [77,78] and vascular genes [79] and used in the treatment of occlusive or stenotic disease. However, no major clinical trials of this are available.

3.1.2. Seeding cells of other sources

Bone marrow and blood cells (progenitor cells) provide an additional source of cells [80] with unique native properties containing many mesenchymal stem cells (MSCs) [81,82]. Researchers have even found cardiac stem cells in the adult ventricle [83]. These cells produced various cytokines resulting in autocrine angiogenicity on seeded ePTFE vascular grafts. It is expected that these pluripotent cells differentiate into cells such as fibroblasts (Fb), ECs and SMCs. Studies have also begun to look at combining different cell
groups in order to optimise the formation of a viable and functional endothelium for example venous [84], adipose tissue and bone marrow [85] and this is discussed later [86].

Until recently, it was thought that vasculogenesis was a purely embryological phenomenon mediated by EPCs but the discovery of undifferentiated EPCs, that originate from bone marrow [80,82,84] and circulate within the vascular tree [87,88] to form tubules and inducing vascularisation [89] has been termed ‘post-natal vasculogenesis’ [90]. While not as pluripotent as stem cells, the ability of these relatively undifferentiated cells to form a neo-network possibly via the vascular endothelial growth factor (VEGF) signalling pathway [91,92] has opened up new possibilities in its clinical application [93]. Recent in vitro studies using seeded-EPCs on either stents or grafts have been shown to promote rapid and complete formation of a neointima [94–96]. On culture, EPCs in the hybrid tissue migrated and proliferated to form a completely endothelialised luminal surface at the stented sites. EPCs are currently harvested from bone marrow, peripheral blood [88] and cord blood [97] using markers for CD-34 [87,94] or AC133 [85].

The number of cells needed to seed a graft is also dependent on the type of the prosthesis graft. For example, ePTFE requires less cells than the honeycomb structured ‘MyoLink’ graft [86,98,99]. Other factors like smoking has been shown to decrease the percentage of seeded cells in these in vitro experiments [48,49]. Using newer innovations like electrospinning, it is now possible to replicate nature’s nanostructures on contemporary biomaterials like poly (lactic-co-glycolic acid) [100].

As far as seeding CABGs are concerned, the ideal sources would be vein or subcutaneous fat using the two-stage seeding. An alternative source of ECs in CABG patients would be mediastinal fat or pericardial fat extracted intra-operatively [101,102]. Using stem cell technology, even regenerative cardiomyocytes from MSCs may be used [103]. This has so far only been evaluated in animals [67]. We are currently working on extraction of ECs from mediastinal fat in a single-stage framework in order to seed 3–4 mm poly(carbonate-urea)urethane (see Fig. 2).

In the current setting, only two-stage seeding has been successful for clinical application. This method however is reserved to teaching hospitals with the necessary culture facilities and because many of these ill patients do not have sufficient time to await surgery. Possible reasons why single-stage seeding has been unsuccessful so far may be that ECs extracted from fat may be sub-optimal with other contaminating cells extracted present [104]. In comparison, ECs extracted from veins are purer. Our current efforts are being concentrated on extracting and purifying ECs from fat whilst

### Table 2

<table>
<thead>
<tr>
<th>Authors</th>
<th>Type of seeding</th>
<th>Clinical use</th>
<th>Source of EC</th>
<th>Number of seeded grafts</th>
<th>Patency/years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meinhart et al. [187]</td>
<td>Two</td>
<td>PVBG</td>
<td>Vein</td>
<td>153</td>
<td>84% at 4 years</td>
</tr>
<tr>
<td>Laube et al. [50]</td>
<td>Two</td>
<td>CABG</td>
<td>Vein</td>
<td>14</td>
<td>91% at 2.5 years</td>
</tr>
<tr>
<td>Deutsch et al. [70]</td>
<td>Two</td>
<td>PVBG</td>
<td>Vein</td>
<td>113</td>
<td>65% at 9 years</td>
</tr>
<tr>
<td>Williams [188]</td>
<td>Single</td>
<td>PVBG</td>
<td>Fat</td>
<td>11</td>
<td>60% at 4 years</td>
</tr>
<tr>
<td>Leseche et al. [189]</td>
<td>Two</td>
<td>PVBG</td>
<td>Vein</td>
<td>21</td>
<td>67% at 6.3 years</td>
</tr>
<tr>
<td>Herring et al. [62]</td>
<td>Single</td>
<td>PVBG</td>
<td>Vein</td>
<td>66</td>
<td>38% at 2.5 years</td>
</tr>
<tr>
<td>Meerbaum et al. [190]</td>
<td>Single</td>
<td>PVBG</td>
<td>Fat</td>
<td>34</td>
<td>42% at 2.5 years</td>
</tr>
</tbody>
</table>

CABG: coronary artery bypass graft; PVBG: peripheral vascular bypass graft.

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**Fig. 2.** Schematic diagram of in vitro reconstruction of hybrid vascular wall. The engineered graft has a hierarchical arterial structure: monolayer oriented HUVEC, RGD covalently bonded onto elastic polymer [8].
removing other contaminating cells without any detrimental effect to the ECs [68]. We hope this may improve graft patency.

3.1.3. Seeding genetically modified cells
An alternative approach to improving seeding efficiency and endothelial function is to use genetically modified ECs [105,106]. This method can promote cell repopulation, negate thrombolytic events and actively secrete anticoagulant peptides. Retroviral gene delivery systems have been employed to deliver a specific gene of interest to the ECs of a developing vessel [105,107]. Another approach has involved generating human EC that constantly overexpresses endothelial nitric oxide synthetase (eNOS) and which inhibits both platelet aggregation and SMC proliferation; two factors most often resulting in IH [108]. Once these initial results were reported, numerous consequent in vitro and in vivo studies showed that it was possible to seed these genetically modified cells via a two-stage technique onto both vascular grafts and stents [77,105,109,110]. These studies have still not progressed onto clinical trials and have raised ethical questions on the role of gene therapy. The latter relates to whether such modifications will be passed onto host progeny and the effects on gene transfer on local homeostatic mechanisms [111]. Moreover, the post-graft implantation retention rates of such transplanted genetically modified ECs have been generally very poor in comparison to normal unmodified ECs. As such, any benefits or potential clinical attributes from enhanced EC function, proliferative ability and viability would be lost due to increased cell detachment on exposure to flow.

3.1.4. Improving cell adherence to the graft lumen
For both tissue engineers and biomaterial researchers, precise control of cell interactions with the prosthetic or biomaterial surface is critical. To this end, the tissue engineer requires the interaction of specific cell types with the material surface used in the vascular construct in order to promote tissue ingrowth as well as regeneration of the construct. In bio-hybrid tissue constructs, the tissue engineer seeks to obtain very specific cells like ECs to place into biomaterial based scaffolds. Optimal cell–polymer interaction would allow the host tissue to integrate with the graft and allow for the development of actual tissue formation.

To this end, the development of bio-hybrid or fully tissue-engineered biological vascular grafts requires the very selective adhesion of actual donor/patient cells onto the implanted vascular graft such that these cells optimally integrate and most importantly adhere onto the surface with limited inflammatory cell-mediated encapsulation.

Numerous research groups have examined whether modifications to the luminal surface of the prosthetic graft can stimulate self-endothelialisation or allow improved adherence of pre-seeded cells when exposed to arterial flow [112]. This lack of adherence as discussed earlier is an important cause of low density seeding; the cause of graft failure so far in clinical trials. The majority of the work on promoting EC adherence and growth to permanent non-biodegradable polymers has involved the modification of the surface with a single coating of endothelial specific adhesion proteins. These substances include albumin [113], albumin–heparin conjugates [114], collagen [115–117], collagen–elastin matrices [118], fibronectin, gelatin [119], fibrin-gelatin [120], laminin [121], extracellular matrix [122], dipryridamole [123], granulocyte-stimulating factor (G-CSF) [124], peptide fragments [125], precolloting with blood, plasma, fibrin glue [126] and serum [127]. Fibrin glue is the commonest substrate used in trials of two-stage seeding in humans [70]. Non-ligand based techniques have included carbon deposition, photo discharge, chemical vapour deposition [128] and plasma discharge technology [129] in order to deposit reactive groups onto polymer surfaces or to directly influence the proteins adsorbed to the surface [130]. These methods to encourage cell attachment have met with limited success owing to the lack of specificity and poor control over protein orientation. More recently, Bura and colleagues developed a poly-electrolyte multilayer on the vessel lumina and showed significant increase in human umbilical vein endothelial cells (HUVEC) adhesion to the construct. This has proved to especially effective when seeding ECs onto hydrophobic surfaces [131].

Physical methods to improve the development of a pseudo-intima on the surface have involved altering the porosity and nano-texture [132] of luminal surfaces to promote tissue ingrowths into the graft and allowing it to act as a three-dimensional scaffold for cell seeding and tissue engineering [116]. Other groups have found that electrostatically seeding ECs on the lumen promotes neo-intimal formation as well [133]. However, the neointima that forms in the lumen of the polymeric graft may not provide comparable physiological properties to a natural endothelium. This limits the integration of the implanted device with the host by this method. Recently there has been an interest in the covalent bonding of short peptide sequences onto the polymer surface of vascular grafts in particular the RGD sequence [134]. We have found similar results (see Fig. 2). An amphiphilic derivative of RGD known as LA-GRGD has been developed in our department [135]. LA-GRGD (200 μg ml⁻¹) has been shown to inhibit tissue factor by >90% comparable to heparin (1 U ml⁻¹) at >90%. Furthermore, cell binding studies showed that LA-GRGD bound 29% of ECs compared with heparin (22%). This finding suggests that LA-GRGD can inhibit fibrinogen binding to activated platelets and promote EC attachment. Overall, RGD based sequences serve as functional ligands for fibronectin which have been shown to promote cell adhesion and attachment [136]. Newer studies have shown the existence of similar fibronectin ligands like the C5 domain [137].

3.1.5. Improving cell adhesion to the graft lumen by using autologous, heterologous and homologous tissues
In the drive to develop hybrid vascular devices, numerous groups have resorted to using autologous tissue extracted from
the patient like fat, veins and bone marrow which do not need to undergo enzymatic digestion [138]. Examples of this specialised work include a recent clinical phase I trial in which loose weave knitted Dacron vascular grafts as used in the infra-popliteal bypass grafts were enhanced by having their luminal surfaces coated with fragmented autologous adipose fat. This study showed a primary patency rate of 67.7% 5 years following implantation [139].

Other autologous biomaterials have also been investigated as potential sources for surface lining of vascular grafts. These include materials as diverse in origin as skin, pericardium, fascia, and the small intestine [140,141]. Autologous materials as vascular vessel substitutes have not proven to be useful as graft materials due to their lack of compliance, particularly under high pressure situations as present within the vascular system. In addition, these exhibit reduced wall stability, thrombogenicity with the propensity to form aneurysms or rupture. However autologous pericardium has proven to be clinically acceptable as a means of reconstructing the right ventricular outflow tract of the heart [142].

Cryopreserved arterial segments used as allografts are another option as they do not require anticoagulation, do not infect easily, are not susceptible to thromboembolisms [143] and have a lesser predisposition to aneurysm formation [144]. The only problem that limits their wider usage are the complications regarding cryopreservation itself, its limited shelf life and a suggestion that SMC proliferation in the tunica media is not promoted [145]. Alternatively, allogenic or human umbilical cord vein grafts have been introduced, but these have never gained wide clinical acceptance [146]. Recently, the concept of an autologous endothelialised vein graft was introduced. In 12 patients undergoing CABGs, the short segments of the patients’ vein were harvested and the human autologous vein endothelial cells (HAVECs) were cultured and subsequently seeded onto acellularised cryopreserved vein allografts. The 3-year primary graft patency rate was shown to be 87% [147].

Xenogenic tissue due to its very nature is seldom used. As such, various research groups have developed approaches of using this material in the decellularised form [147]. Microscopic examination of such decellularised arteries reveals an ECM with correctly oriented collagen and elastin fibrils. Such an ECM can provide the necessary scaffold for seeding or tissue engineering prior to implantation. Since all cellular antigens have been removed, no immune or inflammatory reactions will take place. Wilson and other researchers in this field have shown that this approach may be of greater use in coronary bypass surgeries rather than for vascular transplants since shorter lengths of CABG grafts mean less cells are required for seeding. This is more realistic in the present clinical setting [148,149].

3.2. Tissue engineering of biological grafts

The challenges of tissue engineering blood vessels having the mechanical properties of native vessels with anti-thrombogenic properties are immense [150]. Recent advances indicate that the construction of a tissue-engineered bypass graft that will remain patent in vivo is achievable [151].

Tissue engineering enables the development of fully biological vascular substitutes that restore, maintain and improve tissue function in a manner identical to natural host tissue. Three types of tissue-engineered grafts exist for coronary and vascular bypass: 1) those utilising a biodegradable scaffold [159], 2) those with a bioreistant scaffold such as polyurethane [152], and 3) those with a decellularised matrix tube [153]. The scaffold is then engineered by lining the lumen of the graft either with SMCs, myofibroblasts, chondrocytes or other extracellular basement membranes and seeded with ECs using a bioreactor (Fig. 3) [154] and perfusion culture [116].

The first report in the literature of a fully tissue-engineered vascular vessel construct was reported by Weinberg and Bell [155]. Cultured bovine ECs, SMCs and Fbs were used to construct an artificial graft with the surface cells producing both prostacyclin and von Willebrand factor (vWF). The scaffold used in this case was a Dacron matrix impregnated with collagen. However, the resultant engineered graft was not able to withstand physiological conditions even with the permanent scaffold.

Since then, biological grafts have been developed which can withstand high venous and arterial pressures [156] for up to 6 weeks [157]. The mechanical design of the graft have been improved by cultivating mesenchymal cells (SMCs and fibroblasts) in the presence of l-ascorbic acid in order to produce a three-dimensional ECM with characteristics similar to those observed in vivo. After 56 days of culture, an integrated tubular structure was synthesised, which could be implanted as interposition femoral grafts [158]. The main drawback of this technique is the prolonged culture period.

Blood vessels in polymeric tubes have now also been grown using bovine aortic SMCs placed in a tube shaped resolvable non-permanent polyglycolic acid (PGA) scaffold [159]. This was placed into a silicone tube-based flow circuit which pumps physiological cell culture medium through itself. The graft was seeded 8 weeks later. These pulsed grafts were thicker, had greater suture retention, improved physiological
SMC density together with a higher collagen density. These porcine saphenous arterial xenografts were patent significantly longer than similar ‘non-pulsed’ engineered grafts. Nevertheless, certain problems such as the unpredictability of biodegradable materials, the presence of residual polymer fragments and the possible inflammatory reactions may affect its long-term performance.

A novel way of engineering vascular grafts is by using variable dimension Silastic tubing [160]. This material can be inserted into the peritoneal cavity of an animal where the natural inflammatory reaction covers the latter tubes with myofibroblasts, collagen matrix and MC monolayers [161]. This analogue to vessel structure has remained patent for around 4 months in vivo, during which time, the engineered vessel underwent a transformation into cellular structures that resembled elastic lamellae together with high volumes of myofilaments that had contractile responsiveness to pharmacological agonists.

Recently, a porcine acellular matrix tube has been used for tissue engineering. This is achieved by extracting cells from porcine aorta using trypsin and ethylene diamine tetra-acetic acid (EDTA) [162]. This can then be seeded with ECs derived from human veins. This composite showed good EC attachment and retention. The advantage of this technique is that the acellular matrix will have low immunological potential due to removal of antigens with the potential for use in humans.

Matsuda and co-workers have been investigating the development of hybrid vascular prostheses composed mainly of SMCs, collagen with reinforcement onto polyester fibres. These grafts once developed could then be seeded and have shown promising results [163]. This group has also been evaluating polyurethane and dacron scaffolds [164,165]. Polyurethane scaffolds have been used because of their unique properties in tissue engineering. This scaffold has been either seeded with both SMCs and EC [166] or lined with an artificial membrane composed of collagen and dermatan sulphate before seeding. The SMCs make the scaffold more viscoelastic rather than elastic, thus mimicking the mechanical properties of native vessels. Such grafts were then used in canine models and showed promising results after implantation, with patency rates of up to 75% after 26 weeks [167].

More recently, it has been found that when a template for instance in the form of a string is placed within a co-culture of ECs and SMCs, these cells were found to adhere to and hence form a hollow vessel around this template. SMCs, synthesise collagen and elastin which eventually cross-link to form compliant vessels [168]. In a parallel study to form microvessels, Neumann and co-workers used 80 µm nylon strands as a template onto which SMCs in a culture medium could attach. After a week, this strand was physically removed and the grafts were perfused in a pulsatile flow chamber mimicking the arterial system. After 28 days in culture, these vessels were shown to have 30–40 layers of SMCs evenly distributed throughout the lumen and strong enough to withstand its own weight [169]. However, in the case of larger vessels, it remains to be seen whether such vessels would collapse under their own weight.

Another technique has utilised a polymeric scaffold made up of PGA-polyhydroxyalkanoate (PHA) that was subsequently seeded with a mixture of ECs, SMCs and Fbs [170]. When implanted in vivo for 5 months, no aneurysms were seen and the graft remained patent.

To date there have been very few studies carried out on tissue engineering of cardiac tissue and grafts but one important recent study has emerged by Leor and co-workers who have used novel bio-engineering approaches in order to develop initially cardiac tissue and then coronary bypass grafts utilising three-dimensional alginate scaffolds with fetal cardiac cells [171].

In summary, these studies in coronary and vascular tissue engineering have led to important advances in the progression to a blood vessel substitute that can serve as a living graft. These should be responsive to the surrounding biophysical environment, be self-replicating and have an inherent healing potential. It is now been shown to be possible to develop cellular engineered grafts composed purely of human cells. The mechanical strength of biomaterials derived from the ECM, the production of the matrix and the integrity of the cellular sheets themselves could be significantly enhanced by simple alterations in cell culture conditions. The optimal biological coronary or vascular graft can only be used in vitro if they are incubated within bioenvironmental conditions that they would confront in vivo or during their natural formation. For example, our studies have shown that culturing SMCs within a pulsatile flow model similar to in vivo arterial systems helped mimic nature’s vessels as shown in Fig. 3. Seliktar et al. [172] have discovered that gene expression is enhanced when such constructs are subjected to in vivo cyclic strain. Chello et al. [173] have found that in vitro pressure distension markedly increases expression of adhesion molecules like inter-cellular adhesion molecule (ICAM-1) while earlier studies showed that haemodynamic forces were able to stimulate the expression of platelet-derived growth factor (PDGF), an SMC mitogen [106,174]. Furthermore, the immune reaction can be completely negated by culturing the blood vessel substitutes within the host patient [167]. Therefore, the vascular matrix can be remodelled completely by the body itself to adapt to the local environment, such that autologous cell-based grafts no longer need to be determined by its cellular elements, but also by the local dynamics of the bioreactor. Scientists now attempting to regulate the formation of these grafts with the use of biological response modifiers like ascorbic acid [175].

4. Discussion

Due to the poor patency rate of traditional prosthetic grafts that is primarily due to low compliance and thrombogenicity, seeding and tissue engineering are being used to achieve an internal environment similar to that found in native vessels.
The initial experiments trying to use a single-stage seeding in animals showed good results. However when this was tried in humans the results was poor. It was then realised that animals will spontaneously endothelialise any graft even if not seeded thus explaining the good results seen in animal trials. These poor results in man led to the development of numerous in vitro perfusion systems in order to test luminal endothelialisation of the graft. It was also realised that to get better results in humans, a larger number of cells needed to be seeded. When this was achieved, clinical results improved. However, the inherent problem that exists with seeding is the 2–4 weeks culture time required to produce sufficient cells. Thus, this makes this method unsuitable in the emergency situation. Another way forward would be to accelerate and sustain cell growth in vitro [176].

It was then suggested that extracting EC from fat would allow large numbers of cells to be extracted and that this would be suitable for single-stage seeding procedures. The clinical results of this however were disappointing and may reflect the fact that fat may not be the ideal source for seeding [191].

A completely alternative approach is to develop fully engineered grafts made from a scaffold and mixtures of SMCs/collagen and ECs [177]. This takes weeks to months to incubate in bioreactors before the vessels are indeed ready for implantation [178]. For any planned surgery, such long incubation and culturing times may be appropriate. At the same time, they are inapplicable for any emergency bypass operations. Furthermore, such living unpreserved grafts have a very limited shelf live [179]. Even xeno- or allografts can to some extent be available for constant stock updating, but in the case of autografts, this is simply out of the question [180]. The other problem associated with such fully biological grafts is that they have never undergone any clinical trial. Thus it is unclear how these biological grafts will perform compared to native arteries, veins or the current prosthetic grafts over the long-term [181].

We feel that fully biological tissue-engineered vascular grafts can only replace native vessels when they are grown to outperform the present of vascular grafts in clinical trials [182]. This will take years of research before being realise. What is the current role of the fully biological tissue-engineered blood vessel? In the current scenario, it is limited to the laboratory as an experimental tool that allows further insight into the workings of the vascular system. Innovations such as autologous peritonal incubation as suggested by Campbell et al. [160] that might develop blood vessel conduits free of immune responses may be the next great step forward or another disappointment.

At this stage, we should be looking to create compliant cardiac coronary and vascular conduits with properties to self-endothelialise. This may be possible with the newer generation of polyurethane grafts which will be able to provide an immediate and permanent alternative to native tissue that fully biological and seeded conduits simply cannot match due to their need for prolonged cultures [183]. The next stage in tissue engineering would be to develop arteries that possess both the mechanical and functional qualities that we seek in autologous tissue-engineered vascular replacements but in a form that is available off the shelf, clinically applicable to all centres and cost-effective for surgical use.

Acknowledgments

We acknowledge Dr. Philippe Fernandez and Dr. Murielle Remy-Zolghadri for their useful comments and suggestion in the manuscript. This work supported in part by UCL Biomedica Plc, London, UK and Nervation Ltd., UK who provided a grant to develop bypass graft.

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